Seropositivity and Adverse Birth Events in Migrants From Bilharzia-endemic Areas

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Study Protocol and Statistical Analysis Plan

Project Objectives
The aim of the proposed research project is to study the impact of Schistosomiasis on adverse birth outcomes (ABO) (as defined by low birth weight) in migrants to Europe from *Schistosoma*-endemic areas. This project will examine if pregnancies in *Schistosoma*-infected women are associated with reduced birth weight, premature delivery and stillbirth of the exposed infants.

Purpose:
To examine the association between Schistosomiasis seropositivity and adverse pregnancy outcomes. Hypothesis: In pregnant women from endemic areas for schistosomiasis, positive *Schistosoma* serology is associated with reduced birth weight in the infant.

Rationale:
Schistosomiasis is a widespread helminthic infection, with an estimated 249 million people in 78 countries requiring preventive antiparasitic therapy each year. This infection has a significant association with morbidity worldwide. In most endemic areas long lasting urogenital schistosomiasis constitutes a risk factor for bladder cancer[1,2]. In a study performed in Malawi the reproductive tract was affected in more than 60% of women who excreted *S. haematobium* ova in urine[3]. Transplacental transmission has not been observed, but schistosomiasis of the pregnant uterus has been reported and placental schistosomiasis has been associated with stillbirth[4,5]. Placental schistosomiasis (i.e. detection of schistosomiasis eggs in placental tissue) has been reported only occasionally, but to our knowledge no study has prospectively examined this[5,10,11]. Schistosomiasis has been postulated to be associated with premature delivery and low birth weight[6-9], but further data are required.
Migration to the EU was estimated at 1.7 million people in 2012. Migrants were predominantly from Africa and Asia (25 %, respectively)[12]. In these areas schistosomiasis has an estimated prevalence of 10-20%[13-15]. Therefore there are a large number of migrants from schistosomiasis-endemic areas entering Europe and accessing health care. Many of these migrants are unaware of helminthic infections they may have been exposed to, and the potential outcomes these infections may have.

Methodology:
A prospectively ascertained cross-sectional study using mother-child data pairs will be conducted in pregnant migrants attending for medical care in Rome, Dublin and Jena from *Schistosoma*-endemic areas.
Inclusion criteria: Pregnant women >18 years originally from endemic countries and areas (as defined by WHO) who give written informed consent to the study.
Exclusion criteria: Placental pathology due to any cause and any other medical condition affecting fetal growth.
Primary analysis: Association between *Schistosoma* seropositivity and birth weight (details below).
Secondary analyses: Association between Schistosoma seropositivity and intrauterine growth restriction (IUGR)[16] (defined as birth weight below the 10th birth weight percentile), stillbirth and premature delivery.
Assessments: A questionnaire (see below) will be completed on each subject including demographics, age, medical history, and history of *Schistosoma* infection. Data concerning the primary (birth weight percentiles) and secondary outcomes (intrauterine growth restriction, stillbirth and premature delivery) will be collected. The necessary values consist of gestational age, weight and sex of the new born. Gestational age will be either determined by ultrasound or by calculation of the last menstrual period. Schistosoma-specific antibody detection will be performed locally on serum by a commercially available *in vitro* diagnostics certified qualitative ELISA test.
Questionnaires: Known categorical variables that are affecting fetal growth are smoking, alcohol, diabetes, baby gender, ethnic origin and parity. Known continuous variables are birth weight, gestational length, maternal height and weight[24]. The country of origin will provide the ethnic origin more precisely. Medical conditions and concomitant medication of the mother are documented. Relevant laboratory parameters - if available from the clinical routine – will be added to the dataset: Hemoglobin, Eosinophils, HIV-status and Hepatitis B and C status (Hbs-Ag, Anti-HCV). Factors resulting from exposition that can influence serological results are: previous history of Bilharzia, previous treatment and time since previous treatment of Bilharzia, fresh water contacts in the area of origin or on the way to Europe, time since migration to Europe and the type of travel to Europe. Data will be collected with a standardized case report form (eCRF) and will be pseudonymised at source. Property transfer for material (placenta and serum) is part of the questionnaire.
Sample size estimates: We estimated that n=200 mother-newborn pairs can be realistically ascertained within the funding period in the participating study centers in Germany, Italy and Ireland. Furthermore,

we expect 20% of the serologic tested samples to be positive (i.e. $n_{positive} \approx 40$; $n_{negative} \approx 160$ based on previous literature[13-15]). Intrauterine growth restriction (IUGR) is defined by birth weight below the 10th percentile of the reference curves. It occurs in 5-10% of all pregnancies and can be compared across multi-ethnic populations[16,18-20]. We hypothesize that serologic positivity will result in reduced birth weight. In the given setting of our project, a study with n=200 mother-newborn pairs has a power of 80% to detect a standardized mean difference in birth weight percentiles of 0.5 (in units of standard deviations of a standard normal distribution; a moderate effect according to Cohen) at a significance level α=5% (two-sided; planning by Student's test for 4:1 sample size ratio; nQuery 3.0).

Statistical analysis: The primary analysis will be a Mann-Whitney-Wilcoxon test to compare birth weight percentile distributions between the two groups of newborns born to seronegative and seropositive mothers (at a two-sided significance level α=5%). We will also evaluate known factors that influence birth weight (see above in paragraph questionnaire) in univariate regression models for the birth weight percentile outcome. Single confounders with a potential impact on the relationship between serum-status and birth weight percentile will be included in multiple linear regression models based on the univariate results ($p<0.2$). Similar to these sensitivity analyses, all other inferential statistics to compare the secondary outcomes between the two groups of negative and serum-positive newborns will be done exploratively using Fishers exact tests for the dichotomous variables (IUGR, stillbirth and premature delivery) while reporting the respective effects size estimates and 95% confidence intervals.

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